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(54) Title: <b>ADHESIVE COVER SLIP AND METHOD FOR MICROSCOPY</b>			
(57) Abstract <p>A combination comprising a slide suitable for optical microscopy to which a specimen is applied and an optically transparent cover adhered to the slide over the specimen, wherein the cover comprises: an optically transparent polymeric film having opposite first and second major surfaces; and a contact responsive adhesive on the first major surface and in contact with the slide.</p>			

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## ADHESIVE COVER SLIP AND METHOD FOR MICROSCOPY

Technical Field

- 5 The invention relates generally to the preparation of specimen slides for optical microscopy, and more specifically to a cover for covering the specimen mounted to the slide and a preparation method.

Background of the Invention

- 10 In disciplines such as histology or microbiology where it is common to examine a specimen with light microscopy, it is conventional to mount the specimen on a glass slide. Then, to protect both the specimen and the objective lens of the microscope, it is conventional to cover the specimen with a cover slip. Most cover slips have been made of thin transparent glass. This construction has the disadvantages that such thin glass is  
15 fragile and breaks into sharp pieces, representing a hazard to the user.

- When it is desirable to preserve a specimen on the slide for viewing at a later time, it is known to seal the cover slip to the slide. Solvent-based or melt-based sealing agents may be used to accomplish this. For example, it is known to apply a drop or two of a preparation of resin dissolved in xylene to the slide and specimen before placing the cover  
20 slip onto the slide. When the solvent evaporates, the resin adheres the cover slip to the slide and protects the specimen. This procedure has two disadvantages. Firstly, it takes some time for the solvent to evaporate completely and until that occurs the slide cannot be handled without risking damaging the visualization of the finished slide by smearing the resin. Secondly, it is occasionally necessary to re-stain an old but valuable specimen, and  
25 it is very difficult and time consuming to remove a cover slip adhered in this way without damaging the specimen. Lengthy immersion in solvent, most commonly xylene or toluene, is required, and this is messy and inconvenient.

- An embodiment to the aforementioned method is described in U.S. Patent No. 3,891,327 wherein certain photocurable compositions, including compositions  
30 comprising certain acrylate or methacrylate mixtures and photoinitiators, are used to bond a coverslip to a slide. In the method, a photopolymerizable or photocurable composition is

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applied to the specimen-carrying slide, a cover slip is applied over the specimen and the slide is exposed to an ultraviolet light (UV) radiation source until the photopolymerizable composition has hardened and bonded the coverslip to the slide. The patent states that the method requires five minutes to prepare and UV cure a slide in contrast to the two or three  
5 days required for conventional methods which use prior-known resinous bonding agents and solvent.

In an attempt to ameliorate these known problems, various improvements to the procedure have been introduced. For example, it is known to provide cover slips of polymeric material which are less brittle than those made of glass. To permit the partial  
10 automation of the task of applying cover slips where the volume of work makes it economical to do so, it has more recently been known to provide such polymeric material in the form of a roll that can feed automatically into an applying machine. It is also known to provide a sealing agent as a coating on the roll material, which is then activated in the applying machine, or at the time the cover slip is applied to the slide, by the application of  
15 a solvent. For example, see U.S. Patent Nos. 4,455,188; 4,188,246; 3,939,019; 4,146,414; 4,203,797; 4,853,262; and 3,498,860. Commercially available coverslips of this type are available from Sakura Finetek under the name Tissue-Tek™ Coverslipping Film, and KP Tape available from Klinipath Corporation of Germany. However, it is still not possible to handle a slide prepared in this way immediately, and it remains rather difficult to remove  
20 such a cover slip when restaining of the specimen is required.

U.S. Patent Nos. 4,853,262, 4,188,246; 4,171,866; 4,607,921; 5,580,414; 5,406,421 and 5,590,414, British Patent No. 1,562,643 and German Patent No. 44 03 308 disclose slides, cover slips and/or apparatus for applying cover slips to slides.

## 25 Summary of the Invention

In one aspect, the invention provides a combination comprising a slide suitable for optical microscopy to which a specimen has been applied, and an optically transparent cover adhered to the slide over the specimen; wherein the cover comprises an optically transparent polymeric film with opposite first and second major surfaces, and a contact  
30 responsive adhesive on the first major surface which is in contact with the slide.

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Preferably, a low adhesion backsize is provided on the second major surface of the optically transparent film. Regularly spaced grid lines are also preferably provided along the cover. The grid lines provide a convenient way to gauge the size of specimens or features of specimens. Also preferably, the cover is sufficiently flexible to be capable of being supplied in a substantially continuous roll form.

In another aspect the invention provides a method for preparation of an optical microscopy slide comprising: (a) placing a specimen on a slide; (b) covering the specimen with a cover comprising an optically transparent polymeric film having a contact responsive adhesive on a major surface thereof; and (c) adhering the contact responsive adhesive to the slide.

The method also preferably includes (1) providing covers in the form of a roll of a substantially continuous optically transparent polymeric film having a contact responsive adhesive on a major surface thereof; and (2) separating a cover from the roll before covering the specimen with a cover comprising an optically transparent polymeric film having a contact responsive adhesive on a major surface thereof.

In some embodiments of the invention, a wetting agent can be applied to the cover or the specimen before the cover is adhered to the slide to improve viewing of the specimen. Some compositions suitable for use as wetting agents include xylene, d-limonene, mineral oil, and ethanol.

The invention reduces the disadvantages of the prior art by providing a cover that rapidly adheres to a microscope slide upon application of light pressure, without need of an activating solvent, thereby eliminating the need for long drying or curing times before a slide can be handled. In preferred embodiments of the invention, the cover can be removed from the slide without immersing the slide and cover assembly in a solvent.

#### **Brief Description of the Drawing**

The invention will be further described with reference to the drawing wherein corresponding reference characters indicate corresponding parts throughout the several views of the drawing, and wherein:

Fig. 1 is a top view of a microscope slide with a specimen mounted thereon, sealed in place by a cover according to an embodiment of the present invention;

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Figure 2 is a cross-section side view of the microscope slide and cover of Figure 1 taken substantially along section lines 2-2; and

Figure 3 is a cross-sectional view similar to figure 2 illustrating a second preferred embodiment of the invention.

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#### **Detailed Description of Preferred Embodiments**

Referring now to Fig. 1, microscope slide 10 is shown with specimen 12 mounted thereon. Specimen 12 is seen in this view through the transparent substance of cover 14. In Fig. 2, the same assembly is seen in cross-section side view, and more of the structural details can be made out.

The cover 14 comprises an optically transparent polymeric film 16 having a first side or major surface 18. A layer of contact responsive adhesive 20 is provided on the first major surface 18 of the optically transparent polymeric film 16.

Figure 3 illustrates the cover 14 with an optional layer of an optically transparent scratch resistant coating 24 on a second, opposite side or major surface 22 of the optically transparent polymeric film 16. Above the scratch resistant coating 24 is an optional layer of a low adhesion backsize 26, which helps the cover 14 unroll easily if convenience for the user prompts the cover slip film being delivered as a roll of indefinite or substantially continuous length material. The optically transparent scratch resistant coating can be any optically transparent hard coating known to be compatible and to bond to the optically transparent polymeric films. Many such materials are commercially available.

Preferably, the refractive index of each of the components of the cover 14 are near the refractive index of the glass microscope slide 10. Commercially available glass slides typically have a  $n_D$  of approximately 1.51, and preferred materials for use in the cover have refractive indexes that approximate this value. Accordingly, preferred materials have refractive indexes of at least 1.45, and more preferably of at least 1.47. While materials having a refractive index as high as 1.65 are considered useful, materials with refractive indexes between about 1.47 and 1.55 are most preferred.

Because the total transmission of light through the cover must be adequate to ensure that sufficient light reaches the eye or camera viewing the specimen, the luminous transmittance of light (measured according to ASTM D1003-95) through the cover should

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be maximized. The cover should possess a luminous transmittance of at least about 85%, and more preferably at least about 94%. Accordingly, the luminous transmittance of cover components (e.g., optically transparent polymeric film) should be selected to maximize luminous transmittance and preferably the total transmittance of each component of the cover is at least 85%.

With regard to the optically transparent polymeric film 16, it has been found that thicknesses of between about 100 to 250 microns give the best results. Thicknesses within this range provide good flexibility for use in roll form with automated application systems while maintaining sufficient strength for coating and handling of the film and cover.

While many types of transparent polymeric films could be used, examples of films that are considered suitable include cellulose diacetate, cellulose triacetate, polyethylene terephthalate, styrene-acrylonitrile, and polymethyl methacrylate films. One particularly preferred material is film made from polymethyl methacrylate resin that is commercially available as V045-UVA10-100 from Atohaas Americas, Inc. of Philadelphia, Pennsylvania.

It is presently contemplated that the polymeric film may be activated by a corona, plasma flame or actinic radiation treatment shortly before the application of the layer of contact responsive adhesive in order to improve the bonding between the polymeric film and the contact responsive adhesive. It is also considered to be within the scope of the invention to provide an intermediate or "tie" layer between the polymeric film and the contact responsive adhesive. The selection of an appropriate tie layer material will depend upon the type of contact responsive adhesive and optically transparent polymeric film used in the invention. Many materials known to be useful as tie layers are useful in this invention, for example, chlorine-containing polymers like polyvinyl chloride, vinyl chloride/vinyl acetate copolymers, polyvinylidene chloride, polycarbodiimide and ethylene vinyl acetate polymers and copolymers, acid or anhydride-modified polyethylene, propylene and ethylene vinyl acetate polymers and copolymers may be used in the invention.

The contact responsive adhesives useful in the invention are pressure sensitive adhesives that adhere relatively quickly and strongly to the target surface (i.e., slide specimen mounted thereon) yet exhibit essentially no surface tack to the skin when coated

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on the optically transparent polymeric film. The contact responsive adhesives used in the invention bond to the target surface without need for solvent activation. In contrast, covers prepared with "solvent activated" adhesives will not substantially adhere to the target until the solvent used to dissolve and activate the adhesive is partially or completely evaporated. In the context of this invention, the term "substantially adhere" means that the cover adheres strongly enough to the target to permit handling and use (e.g., viewing ) of the target. Accordingly, a contact responsive adhesive used in the invention should immediately bond and be resistant to shear loading, such that dwell time prior to use of the target is unnecessary or so short it is relatively unnoticeable to the technician preparing the target. However, the adhesive should not bond so aggressively to the target that it cannot be removed from the target without causing significance damage to the specimen.

The precise composition of the contact responsive adhesive may be varied to suit the particular application, however, the contact responsive adhesives coated on the optically transparent film will typically exhibit 180° peel adhesion values of between about 0.1 to 2.8 N/25 mm width, and preferably between about 0.2 to 1.4 N/25 mm width. (The 180° peel adhesion is measured according to the technique described in the Examples.) Adhesives that produce values higher than 2.8 N/25 mm width are generally too adherent to permit removal of the cover without some damage to the specimen. Adhesives that produce values less than 0.1 generally do not adhere sufficiently to the target to remain bonded during handling and storage of the target.

In preferred embodiments of the invention, the contact responsive adhesive will not migrate from the cover to the target when the cover is separated from the target. Most preferably, the adhesive will not flow or creep during storage or block (i.e., form a permanent bond) to the target or to adjacent targets stored in close proximity. Preferably, the contact responsive adhesive, when coated on the optically transparent polymeric film, demonstrates a dynamic shear strength to the target surface (as measured by the test described in WO94/21742) of greater than about 2 kN/m<sup>2</sup>, more preferably greater than about 4 kN/m<sup>2</sup>, and most preferably greater than 6 kN/m<sup>2</sup> at ambient temperature. In some cases, it may be necessary to adjust the coating weight/coating thickness to achieve the desired peel adhesion value. Some of the adhesives useful in the invention may be crosslinked to increase their internal or cohesive strength and reduce peel values to a



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desired level. This is an especially useful technique to use when the contact responsive adhesive comprises BACN or BACNI polymers. Such crosslinking can also help prevent blocking and help maintain a relatively constant peel strength over time.

Preferably, the contact responsive adhesive will substantially retain its advantageous balance of properties throughout extended storage periods. Accordingly, a technician can remove the cover from specimen slides prepared with adhesives, restrain or otherwise rejuvenate the specimen, and adhere a new cover to the slide even after the slide has been stored for an extended period of time (e.g., 2 to 10 years). Such preferred contact responsive adhesives will generally maintain a substantially constant 180° peel adhesion over a long time period (e.g., 5 to 10 years), and will not exhibit significant build-up of 180° peel adhesion over time. Because specimen slides are sometimes stored at relatively high humidity conditions, the preferred contact responsive adhesives will not become solvated, or undergo significant impairment of optical or adhesion properties after contact with water (e.g., up to 24 hours immersion at ambient temperature).

Preferably, the contact responsive adhesive is also resistant to UV and oxidative degradation processes that lead to the discoloration of adhesives that impairs their optical properties. Commonly available antioxidants and UV stabilizers can be added to the contact responsive adhesives to improve their optical and oxidative stability.

Specific examples of polymeric materials comprising or constituting the contact responsive adhesive include: acrylonitrile copolymers (e.g., butadiene-acrylonitrile polymers (BACN polymers), butadiene-acrylonitrile-isoprene polymers (BACNI polymers)); styrene copolymers (e.g., styrene/butadiene/ styrene (SBS polymers), styrene/isoprene/styrene (SIS polymers), and styrene/ethylene/ butylene/styrene (SEBS polymers)); and acrylate copolymers. Blends and mixtures of polymeric materials may be used if desired.

If desired, various other materials may be incorporated into the polymeric material to improve handling or application of the adhesive or to stabilize its optical or bonding properties. For example, tackifiers may be used, although they are generally only preferred for use in adhesives comprising styrene copolymers. Detackifying agents also may be added, although they are less desirable as additives because many detackifying agents (e.g., talc) can impair the adhesive's optical properties. Preferably, such

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detackifying agents will be removed from commercially available polymeric materials if they are present in sufficient amount to impair optical properties. Plasticizers, antioxidants (e.g., organophosphites and phenolics such as those commercially available under the trademark "Irganox" from Ciba Geigy as B561, 1010 and 1076, or from Shell Chemical Company as Ionox 330, or from Bayer Leverkusen as Antioxidant ZKF), UV stabilizers (e.g., hindered amines and benzotriazoles such as those commercially available from Ciba Geigy as Tinuvin 292 and Tinuvin 328) and so forth also may be employed. The exact quantity of such materials may be varied to suit the desires of the compounder, provided that the resulting adhesive possesses the advantageous balance of bonding properties and necessary optical properties. Preferably, only antioxidants or UV stabilizers are combined with the polymeric materials to formulate the contact responsive adhesive used in this invention.

Many useful acrylonitrile copolymers and adhesive formulations based on such polymers are described in the art, for example, U.S. Patent Nos. 5,196,266 and 5,316,849 describe a number of useful copolymers and adhesive formulations. The aforementioned patents are incorporated herein by reference with respect to the polymers and adhesive compositions disclosed therein. The acrylonitrile copolymers should contain less than about 24 wt. %, preferably less than about 20 wt. percent, and most preferably less than about 15 wt. % acrylonitrile units. Particularly preferred among the class of acrylonitrile copolymers are the BACN polymers and BACNI polymers. The BACN and BACNI polymers typically have about 24 to 50 wt. % acrylonitrile units and from about 2 to 20% by weight isoprene units. Preferably, such polymers contain about 20 to 50 wt. % and more preferably about 15 to 50 wt. % acrylonitrile units. Preferably, the BACN or BACNI polymer is compounded with a small amount of antioxidant (less than about 5 wt. % and preferably about 1 to 3 wt. % based on total polymer weight), UV stabilizer (less than about 3 wt. %, and preferably less than 0.5 wt. %, based on total polymer weight) before it is coated on the optically transparent film. It may also be advantageous to further crosslink the adhesive after it is coated on the optically transparent film in order to reduce blocking. To crosslink the adhesive, a small amount of crosslinking agent is compounded with the polymers and other ingredients of the adhesive composition. For example, a small amount of 2-(4-methoxyphenyl)-4,6-bis(trichloromethyl)-1,3,5-triazine can be added

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to promote cross linking. The coated film then can be exposed to an electron beam or high energy light source (such as Sylvania H33CO-400 boosted with a variable power source) until the desired degree of crosslinking is achieved. The energy level required to crosslink the coating will, among other things, depend upon the coating thickness. However, energy levels of about 150-200 millijoules/cm<sup>2</sup> or 1 to 5 Mrads are probably sufficient for most of the coating thicknesses used in this invention. The amount of acrylonitrile monomer in the polymer may also affect the formulation and the extent of crosslinking that is advisable. Accordingly, it may be necessary to conduct some limited optimization of the additive levels and extent of crosslinking for the particular polymer employed. Many acrylonitrile copolymers useful in this invention are commercially available. Examples of such copolymers include polymers available under the trademarks of: "Nipol" and "Zetpol" from Zeon Chemicals Incorporated of Louisville, Kentucky (e.g., Nipol DN-4550, Nipol DN-3380, Nipol DN-3350, Nipol DN-401L, Nipol DN-1201L, Zetpol 2000L, Zetpol 0020, Zetpol 1010, Zetpol 2010L and Zetpol ZSC 2095); "Hycar" from B.F. Goodrich Chemicals Inc. of Richfield, OH (e.g., Hycar 1022); and "Goodyear" from Goodyear Tire and Rubber Co. of Akron, OH (e.g., Goodyear N-206). Adhesive compositions comprising BACN and/or BACNI polymers are presently preferred. Commercially available acrylonitrile copolymers may contain sufficient antioxidants, UV stabilizers and crosslinking agent to eliminate any need to compound the polymeric material with additional stabilizers and crosslinking agents.

Adhesives prepared using styrene copolymers are also useful in the invention. Preferably, such adhesives are prepared using styrene/butadiene/styrene block copolymers (SBS polymers), styrene/isoprene/styrene block copolymers (SIS polymers) or styrene/ethylene/butylene/styrene block copolymers (SEBS polymers). Many useful adhesive formulations based on such polymers are known in the art and some are useful in this invention. For example, U.S. Patent Nos. 5,196,266, 5,316,849 and 5,389,438 describe a number of adhesive formulations based on styrene copolymers and many can be used in this invention. The aforementioned patents are incorporated herein by reference with respect to the polymers and adhesive compositions disclosed therein. Useful adhesive compositions comprise about 20 to 80 wt. % copolymer and correspondingly 20 to 80 wt. % tackifying material wherein the tackifying material is selected from the group

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consisting of tackifier resins (liquid or solid), plasticizers, or blends thereof. Generally, blends of tackifying materials are preferred because the use of blends provides more control over the rheological properties of the adhesive composition than does the use of a single tackifier resin. In this application, compositions based on SEBS or SI block

5 copolymers preferably have a composite mid-block glass transition (CMTg) of about 225 to 240° Kelvin. Compositions based on SBS copolymers preferably have a CMTg of about 215 to 235° Kelvin. Since the adhesion properties of these compositions can be varied by varying the styrene content of the composition (by selection of copolymer and varying the copolymer content of the composition) and the CMTg of the composition, one

10 can vary the types and amounts of components to secure appropriate 180° adhesion values for the contact responsive adhesive. For example, compositions which have copolymer content near the higher end of the range (i.e., 50 to 80 parts by weight) and a CMTg near the higher end of the range or that even exceeds the range (i.e., 240° Kelvin  $\pm$  10° K or 235°K  $\pm$  10°K) which is too tacky can be made useful by slightly increasing the amount of

15 styrene in the composition (e.g., by further increasing the copolymer content). Generally, compositions which contain an amount of copolymer that is at the lower end of the range (i.e., 20 to 45 parts by weight) and a CMTg at the lower end of the range (i.e., 225°K  $\pm$  10°K or 215°K  $\pm$  10°K) have a good balance and adhesion properties for this application. Compositions with copolymer content at the higher end of the range, but a CMTg at the

20 lower end of the range also generally have a good balance of adhesion properties for this application. However, compositions with a copolymer content at the lower end of the range and a CMTg at the higher end of the range or slightly exceeding it will be too tacky for this application. However, the compositions may be able to be slightly reformulated (e.g., by reducing the content of tackifier resin or increasing the composition's styrene

25 content) to adjust tackiness to an acceptable level. SEBS copolymers are the preferred styrene copolymers for use in this invention because they are colorless or nearly colorless, and have good long-term age stability with respect to discoloration of an adhesive. Examples of useful tackifying materials include: tackifier resins made from the polymerization of a feed stream consisting mainly of unsaturated species containing four

30 to six carbon atoms such as "Wingtack" 10, "Wingtack Plus" and "Wingtack" 95 available from the Goodyear Tire and Rubber Company, "Escorez" 1310, available from Exxon

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Chemical Company, and "Hercotack" RT-95, available from Hercules, Inc.; rosin esters and rosin acids such as "Hercoflex" 400, "Hercoflex" 500, "Foral" 85, Regalite" 355 and Permalyn" 305, all available from Hercules, Inc.; mixed aliphatic/aromatic liquid tackifiers such as "Escorez" 2520, available from Exxon Chemical Co.; and polyterpene tackifiers

5 such as "Zonarez" A-25 and "Zonarex" A-100, available from Arizona Chemical Co. and "Piccolyte" HM-85, HM-105, and S-115 available from Hercules, Inc. Also useful are the general class of hydrogenated tackifying resins, including ECR-327, "Escorez" 5380, "Escorez" 5300, "Escorez" 5320, and "Escorez" 5340 all available from Exxon Chemical Co.; "Regalrez" 1018, "Regalrez" 1065, "Regalrez" 1078, "Regalrez" 1094, and

10 "Regalrez" 1126, all available from Hercules Inc.; and "Arkon" P-90, "Arkon" P-100, "Arkon" M-90 and "Arkon" M-100, available from Arakawa Chemical Company; hydrogenated polyterpene resins such as "Nirex" K-85, "Nirez" K-105 and "Nirez" K-110, available from Reichhold Chemical, Inc.; and hydrogenated aliphatic and aliphatic/aromatic resins such as ECR-142H and ECR-143H, available from Exxon

15 Chemical Company. Preferred tackifying resins include the aliphatic hydrocarbon resins, the hydrogenated resins and the polyterpene resins. Especially preferred are the hydrogenated aliphatic and the hydrogenated aliphatic/aromatic hydrocarbon resins. The liquid plasticizer oils suitable for use in the adhesive of the invention include naphthenic oils such as "Shellflex" 371, available from Shell Chemical Co., paraffinic oils, aromatic

20 oils, and mineral oils such as "Kaydol" oil, available from Witco Chemical Corp. Preferred liquid plasticizers include naphthenic oil and mineral oil. Additionally, small amounts of antioxidants and UV stabilizers are advantageously compounded into the composition prior to coating of the adhesive composition. Examples of commercially available styrene copolymers useful in the invention include: SIS copolymers sold under

25 the trademark "Kraton" by Shell Chemical Company, such as 1107 and 1111, those sold under the trademark "Enichem" by Enichem U.S.A. such as SOL T, those sold under the trademark "Quintac" by Nippon-Zeon such as 3430 and 3530, those sold under the trademark "Finaprene" by Fina Chemical Company such as 424; SBS copolymers such as "Kraton" 1101 and "Kraton" 1102, those sold under the trademark "Stereon" by Firestone

30 Synthetic Latex and Rubber Company such as 840A, "Enichem" Sol T 1205 and "Enichem" Sol T 161 C; and SEBS copolymers "Kraton" 1657, "Kraton" 1654X,

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"Kraton" 1652, "Kraton" 1651, and "Kraton" 1650. Especially preferred are the SEBS copolymers.

Suitable acrylate copolymer adhesives for use in this invention include those comprising units derived from about 20 to 100 wt. % of a mixture of (a) at least about 20 to 50 wt. % of an acrylate ester of a C<sub>4</sub> to C<sub>12</sub> alcohol, wherein a homopolymer of the acrylate ester would have a glass transition temperature (T<sub>g</sub>) less than about -50°C; (b) about 0 to 50 wt. % of one or more nonpolar or mildly polar acrylate, methacrylate or substituted vinyl comonomers, wherein a homopolymer of each such comonomer used would have a T<sub>g</sub> of about -50 to -20°C; and (c) about 42 to 70 wt. % of one or more nonpolar or mildly polar acrylate, methacrylate or substituted vinyl comonomers, wherein a homopolymer of each such comonomer used would have a T<sub>g</sub> greater than about 20°C; with about 0 to 80 wt. % of a nonpolar or mildly polar acrylate, methacrylate or substituted vinyl comonomer wherein a homopolymer of each comonomer used would have a T<sub>g</sub> of about -20°C to 20°C. Such copolymers may also contain additional units derived from, for example, chain transfer agents, acrylamide functional disubstituted acetyl aryl ketone photoinitiator and so forth depending upon the method used to polymerize the copolymer. Representative examples of monomers of type (a) include: n-butyl acrylate, 2-ethylhexyl acrylate, isooctyl acrylate and isononyl acrylate. Representative examples of monomers of type (b) include ethyl acrylate and phenoxyethyl acrylate. Representative examples of monomers of type (c) include isobornyl acrylate or N-vinyl caprolactum. The acrylate copolymers can be made according to various known methods. Examples of useful methods are described in U.S. Patent Nos. 5,654,387, 5,728,786, 5,616,670, 5,202,361, 5,840,783, 5,648,425, and 5,506,279, which descriptions regarding polymerization are incorporated by reference herein. The 180° peel adhesion of such adhesives can be further modified through crosslinking (to increase internal cohesiveness) and additives such as plasticizers. Detackifiers should be avoided when their use would impair the optical properties of the adhesive.

While covers according to the invention may be provided pre-cut to a size suitable for use, it is also possible to deliver the cover in the form of a roll of indefinite length material for automated application. When this is done, an optional low adhesion backsize is presently contemplated to be desirable to minimize the force needed to unwind the roll.

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Coatings useful in preparing low adhesion backsizes are known in the art. Examples of particularly preferred materials include coatings comprising urethane, acrylic, silicone and fluorocarbon polymers and many such coatings are commercially available. Examples of suitable coatings include 100% solids, UV cured, platinum catalyzed silicone system  
5 known as "6200" or "8000" silicone series from DCP-Lohja of Westchester, IL.

Alternatively, a release liner may be provided to minimize the force needed to unwind the film. A polyester release liner prepared with the above platinum catalyzed silicone layer, commercially available from DCP-Lohja of Westchester, IL is currently considered suitable.

10 The contact responsive adhesive composition can be coated on the optically transparent polymeric film using conventional techniques, such as solvent coating by methods such as reverse roll, knife-over-roll, gravure, wire wound rod, floating knife or air knife, hot-melt coating such as by slot orifice coaters, roll coaters or extrusion coaters, at appropriate coating weights. Alternatively, a UV activated initiator may be included and  
15 the monomer mixture may be partially cured by UV exposure into a viscous liquid. This viscous liquid can be coated out by any conventional technique. Final properties are developed by a second UV exposure. U.S. Patent No. 5,230,701 (Meyer et al.), which is herein incorporated by reference, discloses suitable adhesive coating methods. The adhesive may be first coated on a liner material, using one of the aforementioned  
20 techniques, and then laminated to the optically transparent film (e.g., using a roller to apply pressure) to form the cover. The liner can then be used as a carrier for the cover or it can be removed. If desired, the adhesive composition can be subjected to a cure step after coating (e.g., by UV radiation or electron beam radiation) to crosslink the coating. Preferably, adhesive coatings of about 13 to 51 microns are used in this invention.

25 It may be desirable to print indicia on the cover slip film, especially in the form of grid lines with a predetermined spacing. This permits the viewer of the specimen to gauge the size of the specimen, or to count objects within an area.

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Examples180° Peel Adhesion

180° Peel adhesion was measured essentially according to ASTM D3330 using a model SP101B testing machine with type SPM-04 modifications (commercially available from IMASS of Hingham, MA), except that a glass target surface and peel rate of 229 cm/min were used.

Refractive Index

The refractive index was measured using an ABBE-3L refractometer (Bausch & Lomb, Rochester, NY) or a Metricon Model 2010 Prism Coupler (Metricon, Pennington, NJ).

Example 1

A contact responsive adhesive formulation was prepared by placing 30.2 liters of methylethylketone into a 38 liter Ross mixer and stirring at 20 rpm using only paddles. While stirring, 8.2 kilograms BACNI polymer (Nipol DN 1201L from Zeon Chemicals of Louisville, KY) was added in approximately ¼ kilogram blocks. At 24 and 48 hours the mixing paddles were raised and clumps of the polymer attached to the paddles were knocked off. The mixture was stirred for a total of 76 hours at approximately 23°C.

The adhesive was then coated onto a release liner using a knife coater with a 356 micron wet gap. The coated construction was dried at 65°C to obtain a 50 micron coating. The release liner was a 35 micron thick polyester terephthalate film coated on one side with a silicone release coating and coated on the other side with an anti-blocking agent.

The release liner coated with adhesive was then fabricated into a cover tape by laminating the adhesive-coated release liner onto a 76 micron thick, oriented polymethylmethacrylate film. Orientation was imparted to the film by stretching the film 2.25 times in the machine direction and 2.3 times in the cross direction.

The refractive index of the adhesive was measured and found to be 1.515. The 180° peel adhesion of the cover to glass was found to be 0.28 N/25 mm width, an acceptable value for the invention.



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Test strips were prepared from the cover tape by cutting pieces of 20 cm x 2.54 cm in size. These pieces were hand laminated to glass plates using a 3.8 cm diameter rubber hand roller and glass. In some of the examples, a wetting agent was applied to the glass plate at the rate of two drops per 3 cm of tape length before lamination. The 180° peel adhesion of the laminates was then measured both initially, and at various times after lamination for two wetting agents. The 180° peel adhesion data, and wetting agents are presented in Table 1.

Table 1

Wetting Agent	Initial 180° Peel Adhesion (N/25 mm)	Day 1 180° Peel Adhesion (N/25 mm)	Day 4 180° Peel Adhesion (N/25 mm)	Day 7 180° Peel Adhesion (N/25 mm)
None	0.28	0.81	0.83	0.89
d-limonene	0.028	0.22	0.14	0.22
Xylene	0.056	0.083	0.083	0.11

10

The data shows wetting agents can lower the initial 180° adhesion values. However, they rapidly improve over time. As will be described later, the cover demonstrates excellent initial resistance to shear loads. This indicates even when wetting agents are used. Accordingly, even though the initial peel adhesion may not immediately be optimum, slides with the cover can be handled immediately. The specimens were inspected by eye with a microscope under high magnification, and the subjective image quality was found to be excellent.

Further test strips were prepared from the cover tape in order to test the resistance of a cover to a shear load when a wetting agent is used. Additional 24 mm x about 75 mm test strips were prepared and hand mounted onto glass microscope slides containing three drops of a wetting agent. The slides were then over-rolled with a 2.0 kg rubber roller and allowed to dwell for a specified period. The resistance of the cover-slide bond to a shear load was evaluated by measuring the ease with which the cover could be displaced along the slide by first supporting the slide with the thumb on the side carrying the cover and four fingers on the opposite side and then applying a shear force with the thumb,

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"upwards" and downwards" along the cover to promote slippage or failure. The resistance of the cover to move along the slide was scored are as follows:

- No stick or easy slippage with minimum thumb shear force
- 0 Slippage with firm thumb shear force
- 5 + No slippage with hard thumb shear force.

The resistance scores for various dwell times and wetting agents are presented in Table 2.

**Table 2**

Wetting Agent	Shear force (0 dwell)	Shear force (5 min. dwell)	Shear force (60 min. dwell)	Shear force (24 hour dwell)
Xylene	+	+	+	+
d-limonene	+	+	+	+

- 10 The data shows that the covers possessed good resistance to shear loads even without any dwell time.

- The luminous transmittance and haze of the cover was measured. The method described in ASTM D1003-95 was used to measure transmittance and haze. Haze-Guard™ Optical Tester (available from BYK-Gardner USA of Silver Spring, MD) was used to carry out the measurements. The % luminous transmittance was 94.8%, the haze was 2.32%, and the clarity was 99.6%.
- 15

Example 2

- 20 This example illustrates the preparation of an optically transparent film with a scratch-resistant coating. A 0.5 m length of 6 inch wide PMMA film was coated with a hydantoin hexacrylate solution (20% in toluene), containing Irgacure® 1173, commercially available from Ciba Specialty Chemicals of Tarrytown, NY) in an amount of 4 parts by weight to 100 parts of hydantoin hexacrylate. The scratch-resistant coating
- 25 was done in a drawdown fashion with a #7 Meyer rod. The coating was allowed to dry and was UV cured under a F300 lamp (from Fusion UV Systems of Gaithersberg, MD), at 50 fpm. Coating thickness was about 2.5 microns. The coating adhesion to the substrate

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was tested according to ASTM D3359 resulting in a value of 5. The coating was tested for hardness according to ASTM D3363, resulting in a 4h hardness rating.

### Example 3

5 This example illustrates the preparation of another optically transparent film with a scratch-resistant coating. A 0.5 m length of PMMA film 15 cm wide was coated with Gafgard® 233, commercially available from International Specialty Products of Wayne NJ, mixed with Esacure KB1, commercially available from Sartomer of Exton, PA, in an amount of 4 parts by weight to 100 parts of the monomer. Coating was done in a  
10 drawdown fashion with a #3 Meyer rod. The coating was UV cured under a F300 lamp at 100 fpm. Coating thickness was measured as about 9 microns. The coating adhesion to the substrate was tested according to ASTM D3359 resulting in a value of 5. The coating was tested for hardness according to ASTM D3363, resulting in a 3h hardness rating.

### Example 4

15 This example illustrates the preparation of a coverslip film comprising a contact responsive adhesive containing ultraviolet light stabilizers. The adhesive was prepared using a similar procedure to that described in Example 1 except that 0.29 grams of Tinuvin 292 (Ciba Specialty Chemicals) was added to 79.1 grams of the adhesive  
20 composition prepared in Example 1. The resulting mixture was stirred until uniform. A cover tape was prepared using a procedure similar to that described in Example 1 except that the new adhesive composition was used and the coating thickness was 71 microns. The refractive index of the tape was 1.514 and the 180° peel adhesion of this coverslip tape to glass was 1.45 N/25 mm wide.

25

### Example 5

A stabilized contact responsive adhesive composition was prepared by adding 0.21 grams of Tinuvin 292 and 0.578 grams of Tinuvin 328 (both commercially available from Ciba Specialty Chemicals) to 87.16 grams of the adhesive of Example 1. Once again, the  
30 mixture was stirred until uniform. The resulting composition was used to prepare a cover tape using a procedure similar to that described in Example 1 except that the new adhesive

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was used and the coating thickness was 64 microns. The refractive index of the adhesive was 1.5169 and the 180° peel test value of the cover tape to glass was 0.70 N/25 mm wide.

Examples 6 to 8 and Comparative Examples C1 to C2

- 5           Adhesive formulations were prepared by blending an SEBS block copolymer with hydrogenated mixed aromatic resin (Regalite® R91, Hercules, Incorporated, Wilmington, DE, T<sub>g</sub> = 306°K) and mineral oil (Mineral Oil U.S. Patent No., Paddock Laboratories Inc., Minneapolis, MN, T<sub>g</sub> = 199°K) and dissolving the mixture in toluene to yield an approximately 20 wt. % solution. The resulting solutions were coated directly onto 76
- 10   micron thick polyester terephthalate films using a knife coater with a 259 micron wet gap. The coated constructions were dried at 65°C. The solutions were also coated in the same manner directly onto 76 micron thick, oriented polymethylmethacrylate film for optical testing. The orientation was imparted to the polymethylmethacrylate film by stretching the film 2.25 times in the machine direction and 2.3 times in the cross direction. The
- 15   formulations, optical properties, coating thicknesses and 180° peel adhesive values are presented in Table 3.

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Table 3

Example	SEBS (wt. %)	Regalite P91 (wt. %)	Mineral Oil (wt. %)	Refractive Index	Transmittance (%)	Haze (%)	Clarity (%)	Additive caliper (microns)	180° Peel Test (N25 mm wide)	CMTg (°C)
6	70 <sup>1</sup>	18	12	1.4907	95.5	0.74	99.1	41	2.6	226
7	72 <sup>2</sup>	26	2	1.5126	95.4	0.47	99.7	41	2.0	237
8	44 <sup>2</sup>	25	31	1.5015	95.5	0.49	99.2	46	0.3	228
C1	68 <sup>1</sup>	30	2	1.5006	95.5	0.49	99.6	30	14.0	237
C2	44 <sup>2</sup>	37	19	1.5089	95.3	0.50	99.2	28	9.1	242

<sup>1</sup> Kraton G1657<sup>2</sup> Kraton G1652M

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The data shows that the 180° peel adhesion value for compositions based on SEBS copolymer may be sensitive to the amount of tackifier resin used in the compositions. Example 6 and Comparative Example C1 and Example 8 and Comparative Example C2 have similar amounts of copolymer and similar CMTgs. However, the 180° peel adhesion in the composition of Comparative Example C1 are unacceptable for this application. However, the 180° peel adhesion probably could be improved to an acceptable level by increasing the copolymer content or decreasing the amount of tackifier resin.

Examples 10 to 12 and Comparative Example C-4

Adhesive formulations were prepared by solvating a BACN polymer in methylethylketone solvent. This adhesive solution was prepared at 25 wt% solids and coated onto a release liner 50 micron thick polyester terephthalate film coated on both sides with a silicone release coat using a knife coater with a 127 micron wet gap. The coated construction was dried at 65°C and the coated release liner was laminated to a 76 micron thick, oriented polymethylmethacrylate film (orientation was imparted by stretching the film 2.25 times in the machine direction and 2.3 times in the cross direction). The acrylonitrile copolymer, % acrylonitrile monomer in the copolymer, Mooney viscosity of the copolymer, refractor index of the adhesive compound, total transmittance, haze, clarity, coating thickness and 180° peel adhesion values are presented in Table 4.

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Table 4

Example	BACN <sup>1</sup>	Acrylonitrile (%)	Mooney Viscosity	Refractive Index	Transmittance (%)	Haze (%)	Clarity <sup>2</sup> (%)	Adhesive Caliper (microns)	180° Peel Adhesion (N/25 mm width)
C4	DN-401LL	18.5	38	1.5184	95.0	0.79	99.8	51	High
10	DN-3350	33	50	1.5169	94.9	1.11	99.7	51	0.3
11	DN-3380	33	80	1.5172	94.2	3.39	99.4	58	0.5
12	DN-4555	45	55	1.5168	95.2	1.45	99.7	51	0.4

<sup>1</sup> copolymers from Zeon Chemicals Incorporated, Louisville, KY, sold under trademark "Nipol".<sup>2</sup> clarity is a measure of the amount of light in percent that is not scattered at an angle of less than 2.5°.

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Examples 13 - 26 and Comparative Examples C5 - C8

A series of experiments were performed to determine whether acrylate adhesives could be prepared which would have the requisite properties for use with the present invention. A number of compositions were made by first partially polymerizing a mixture of monomers according to Table 5. For each 100 parts by weight of monomer 0.04 pbw of Irgacure® 184, commercially available from Ciba Specialty Chemicals of Tarrytown, NY, was added. The partial photopolymerization was accomplished using a bank of 40-watt fluorescent blacklights to provide a coatable syrup.

Following the partial polymerization, an additional 0.16 pbw of Irgacure 184 was added to the resulting syrup. This mixture was knife coated between two layers of 51 micron thick poly(ethylene terephthalate) (PET) film that were coated with premium silicone release coatings. Coating calipers were about 51 microns. The coating was exposed to a bank of blacklight bulbs with an intensity of 2.0 mW/cm<sup>2</sup> for about 5.0 minutes providing an irradiated dose of about 556 mJ/cm<sup>2</sup>. The described procedure is similar to some of the Examples (particularly Example 9) of U.S. Patent No. 5,506,279, which is hereby incorporated by reference.



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Table 5

Example	%IOA	%IBA	%PEA	%NVC	2-EHA	Refractive Index	Peel Adhesion
C5	12.5	75	12.5	0	0	1.5080	0
C6	20	50	30	0	0	1.5090	0
C7	0	50	50	0	0	1.5265	0
13	0	20	80	0	0	1.5461	0.06
14	0	30	0	24.5	45.5	1.4996	0.14
15	30	25	45	0	0	1.5122	0.17
16	25	50	25	0	0	1.5081	0.22
17	0	30	0	21	49	1.4970	0.31
18	0	20	0	28	52	1.4975	1.17
C8	20	0	80	0	0	1.5382	1.53
19	0	20	0	24	56	1.4950	2.0
20	0	10	0	27	63	1.4828	High
21	0	10	0	31.5	58.5	1.4959	High
22	50	0	50	0	0	1.5080	High
23	50	0	50	0	0	1.5080	High
24	37.5	25	37.5	0	0	1.5080	High
25	40	0	60	0	0	1.5150	High
26	10	75	15	0	0	1.5166	High

Table Legend

- IOA            iso-octyl acrylate  
 5    2-EHA        2-ethylhexyl acrylate  
      IBA        isobornyl acrylate  
      PEA        phenylethyl acrylate  
      NVC        n-vinyl caprolactam

- 10    It will be observed that some of the compositions had no peel adhesion to glass, while some had high tack which took them out of the range of acceptable 180° peel adhesion values for contact responsive adhesives and made them too aggressive with respect to contacting delicate microscopic specimens. While the 180° peel adhesion value for Example C8 appears to be in the acceptable range, it actually was not. The sample

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provided a "shocky" peel or exhibited stick-slip phenomena. When the 180° peel adhesion of the sample measured again at a peel rate of 30 cm/min., 180° peel adhesion value was 5.6 N/25 mm width and the sample adhered very strongly to the slide.

As various changes could be made in the above constructions and methods without departing from the scope of the invention as defined in the claims, it is intended that all matter contained in the above description or shown in the accompanying drawings be interpreted as illustrative and not in a limiting sense.

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We Claim:

1. A combination comprising a slide suitable for optical microscopy to which a specimen is applied and an optically transparent cover adhered to the slide over the specimen, wherein the cover comprises:
  - an optically transparent polymeric film having opposite first and second major surfaces; and
  - a contact responsive adhesive on the first major surface and in contact with the slide.
2. The combination according to claim 1, wherein the optically transparent polymeric film is selected from the group consisting of polyester, cellulose diacetate, cellulose triacetate, styrene-acrylonitrile, and polymethyl methacrylate films.
3. The combination according to claim 1, wherein the contact responsive adhesive composition comprises polymer that is selected from the group consisting of acrylonitrile copolymers, styrene copolymers, and acrylate copolymers.
4. The combination according to claim 1, wherein the cover further comprises a low adhesion backsize on the second major surface of the optically transparent polymeric film.
5. The combination according to claim 4, wherein the low adhesion backsize is a coating comprising polymer selected from the group consisting of urethane, acrylic, silicone and fluorocarbon polymers.
6. The combination according to claim 5, wherein the cover is sufficiently flexible to be capable of being supplied in continuous roll form; and the optically transparent film has a thickness of less than approximately 0.25 mm, and a total transmission of at least 85%.

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7. The combination according to claim 6, wherein the optically transparent film and contact responsive adhesive have refractive indexes of at least 1.45.

8. The combination according to claim 7, further comprising regularly spaced grid lines along the optically transparent film.

9. The combination according to claim 6, wherein the cover further comprises a low adhesion backsize on the second major surface of the optically transparent polymeric film.

10. A method of preparing a specimen for optical microscopy, the method comprising the steps of:

- (a) placing the specimen on a slide;
- (b) covering the specimen with a cover comprising an optically transparent polymeric film having a contact responsive adhesive on a major surface thereof; and
- (c) adhering the contact responsive adhesive to the slide.

11. The method according to claim 10 further comprising the following steps: providing covers in the form of a roll of continuous optically transparent polymeric film having a contact responsive adhesive on a major surface thereof; and separating a cover from the roll before the step (b) of covering the specimen with a cover comprising an optically transparent polymeric film having a contact responsive adhesive on a major surface thereof.

12. The method of claim 10 wherein the optically transparent polymeric film is selected from the group consisting of polyester, cellulose diacetate, cellulose triacetate, styrene-acrylonitrile copolymers and poly methyl methacrylate films, and the contact responsive adhesive comprises polymer selected from the group consisting of acrylonitrile copolymers, styrene copolymers or acrylate copolymers.

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13. The method according to claim 10 further comprising the following step:  
transmitting through the cover at least 85% of light incident on the cover.

14. The method according to claim 10 further comprising the following step:  
5 after step a, applying a wetting agent to the specimen or cover before covering the  
specimen with the cover.

15. The method according to claim 14 wherein the wetting agent is selected  
from the group consisting of xylene, d-limonene, mineral oil, and ethanol.

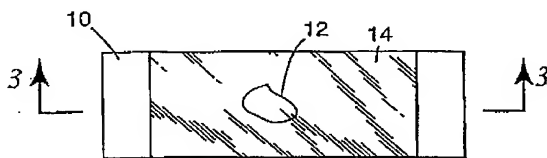
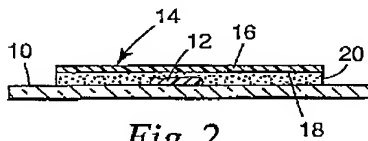
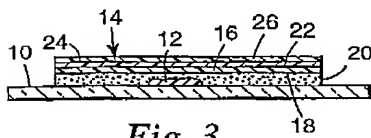
10 16. The use of a tape comprising an optically transparent polymeric film having  
opposite first and second major surfaces and a layer of contact responsive adhesive on the  
first major surface of the cover for an optical microscopy slide.

15 17. An optically transparent cover for covering a specimen on a slide for  
optical microscopy, the cover comprising:  
an optically transparent polymeric film having a first and second major surface;  
and  
a contact responsive adhesive on the first major surface of the optically transparent  
20 film.

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*Fig. 1**Fig. 2**Fig. 3*

## INTERNATIONAL SEARCH REPORT

In tional Application No  
PCT/US 99/07800

A. CLASSIFICATION OF SUBJECT MATTER  
IPC 6 G02B21/34 G01N1/31

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)  
IPC 6 G02B G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the International search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	FR 2 542 876 A (JOUET CIE GLE) 21 September 1984 (1984-09-21) abstract; figure 2 page 1, line 13 - page 1, line 21 page 2, line 16 - page 3, line 8 ---	1, 10, 16, 17
X	CH 400 607 A (WALTER JOKOB MERZ) 30 April 1966 (1966-04-30) page 1, line 47 - page 1, line 60 figures 1,2 ---	1, 10, 16, 17
A	US 4 077 830 A (FULWILER STANLEY S) 7 March 1978 (1978-03-07)  abstract: figures 1,2 column 3, line 8 - column 4, line 19 --- -/-	1-7, 9-13, 16, 17

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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Date of the actual completion of the international search

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## INTERNATIONAL SEARCH REPORT

In International Application No.  
PCT/US 99/07800

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevance to claim No.
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Form PCT/ISA/210 (continuation of second sheet) (July 1992)

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## INTERNATIONAL SEARCH REPORT

Information on patent family members

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